UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: December 15, 2015

SUBJECT: Acetochlor: Review and Evaluation of an Acute, Sub-chronic Neurotoxicity, and a 28-Day Inhalation Toxicity Studies in Rats.

PC Code: 120601
Decision No.: 7314
Petition No.:
Risk Assessment Type:
TXR No.: 0057234
MRID No.: 45357501, 45357502 & 49031001 45811002, 45811003

DP Barcode: D274337/272416/412326
Registration No.:
Regulatory Action:
Case No.:
CAS No.:
40 CFR:

Ver.Apr. 2010

FROM: **Ayaad Assaad, D.V.M, Ph.D.** 12/12/2015
Toxicologist, RAB IV
Health Effects division (7509P)

THROUGH: **Elissa Reaves, Ph.D.,** 12/15/15
Branch Chief, RAB IV
Health Effects Division (7509P)

TO: **Reuben Baris/Maggie Mrudick**
Herbicide Branch
Registration Division (7505P)

I. **CONCLUSIONS:** RAB 4 has reviewed the following toxicology studies available for acetochlor, an acute and subchronic toxicity studies and an inhalation study in rats. These three studies have been used in the hazard characterization of the chemical, and the Data Evaluation Records [DERs] are appended. The toxicology database for acetochlor is considered complete. Data are sufficient for selecting endpoints for all exposure scenarios and for FQPA evaluation. There is also a complete mutagenicity battery. There are no data gaps at this time.

II. ACTION REQUESTED: None

III. BACKGROUND:

The registrant, Monsanto Company, submitted one, 28-day study for alachlor in rats to bridge and complement acetochlor toxicology database, one acute neurotoxicity study in rats, and one subchronic neurotoxicity in the rat with Acetochlor for review and preparing a standard DERs for these studies. The DERs are attached and executive summaries are presented below:

IV. RESULTS/DISCUSSION (or MRID Summary Table, etc.)

1. 28-day subchronic inhalation study in the rat:

EXECUTIVE SUMMARY: In a whole-body inhalation toxicity study (MRID 49031001) Alachlor (Lot No. MDLT 0801 B; 94.3% purity) was administered as an aerosol to male and female Sprague-Dawley rats (15/sex/concentration) for 6 hours/day, 5 days/week for 4 weeks at mean analytical concentrations of 0, 0.06, 0.22, or 0.51 mg/L. The MMADs (mass median aerodynamic diameters) were 2.3, 2.1, and 2.2 μm for the low-, mid- and high concentrations, respectively.

All animals survived until scheduled sacrifice. Redness around the nose and mouth was observed in males and females in the mid and high exposure groups. Red discharge around the nose, mouth, and to some extent near the eyes was observed following each exposure in low incidence at the low concentration, but was evident in all animals at the mid and high concentrations. Salivation was observed in all animals following exposure at the mid and high concentrations throughout the experimental period. Statistically significant lower body weight was observed in males at the high concentration on days 15 and 22, and in females at the mid and high concentration beginning on day 8 or 15 and continued through the end of the experimental period. Lower mean platelet counts were noted in males at each exposure concentration in a dose-related manner, but statistically lower only at the high concentration. Statistical increases in the red blood cell count, hematocrit, and hemoglobin concentration were observed in males at the low and high exposure, but not at the mid exposure concentration. Glucose concentrations were lower in males at the high exposure and in females at the mid and high exposure. Absolute kidney weight at the mid concentration and kidney relative-to-body weight at the mid and high concentration were statistically higher in males. Kidney relative-to-body weight at the high concentration was statistically higher in females. Absolute and relative liver weights were statistically higher in males at the high exposure concentration. Relative liver and brain weights at the high concentration were statistically higher in females. No test material-related microscopic lesions were observed.

Based on the effects seen in this study, a NOAEL in male and female Sprague-Dawley rats was the aerosol concentration of 0.06 mg/L. A LOAEL in male and female rats was the aerosol concentration of 0.22 mg/L based on clinical signs (redness around the nose, mouth, and to some extent eyes was observed in males and females and salivation) and organ weight changes (liver and kidney). Body weight decreases although statistically significant were less than 6%.

reduced food consumption (males and females), increased incidence of clinical signs during the FOB, and decreased motor activity in females at the time of peak effect, with a NOAEL of 500 mg/kg bw/day.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

III. Subchronic neurotoxicity study in rats:

EXECUTIVE SUMMARY: In an oral subchronic neurotoxicity study (MRID 45357502), Acetochlor (tech., 94.7% a.i., batch/lot # P11) was administered in the diet to 12 Alpk:AP_fSD rats/sex/group at dose levels of 0, 200, 600 or 1750 ppm (equivalent to 0, 15.4, 47.6 or 139.0 mg/kg bw/day, males and 0, 18.3, 55.9 or 166.5 mg/kg bw/day, females) for 93 days. A neurobehavioral assessment (functional observational battery and motor activity testing) was performed in all animals/sex/group at -1 week pretest and at weeks 2, 5, 9 and 14. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Brain, spinal cord and peripheral nervous system of the control and high dose animals were examined microscopically; brain weights were also measured.

At 1750 ppm, slight but statistically significant decreases in mean body weight (2.6 to 4.1% less than controls) and weight gain (↓14 to ↓20%, males and ↓25% to ↓30%, females) were reported in both sexes in the early weeks of the study. Decreases thereafter were not significant but continued throughout the study and at termination, mean body weight/weight gain was decreased by 5.4%/11.3% in males and 3.3%/9.8% in females at study termination. During the FOB evaluations at week 2, but not at later times, a statistically significant decrease in hindlimb grip strength (↓44%) in males was observed, these decreases in FOB in week-2 were not considered treatment related since it was not detected in weeks 5, 9 or 14 or in females at any dose level or any measurement interval. There were no treatment-related increases in clinical signs of toxicity nor effects on other neurobehavioral parameters in the FOB, motor activity, brain weight or gross/microscopic neuropathology. **The LOAEL is not observed. The NOAEL is 1750 ppm (139.0 mg/kg, the highest dose tested).**

The study is classified as Acceptable (Guideline) – and satisfies the guideline requirements for a subchronic neurotoxicity study in rats (870.6200b).

MRID Summary Table Example

Study Type	MRID	Comments
28-day inhalation study (rats)	49031001	Acceptable/guideline
Acute neurotoxicity study (rats)	45357501	Acceptable/guideline
Subchronic neurotoxicity study (rats)	45357502	Acceptable/guideline

This 4-week subchronic inhalation toxicity study in the rat is **Acceptable (Guideline)** and satisfies the Guideline requirement for a subchronic inhalation study in the rat (OCSPP 870.3465; OECD 413). A list of deficiencies is given at the end of this document, but it is noted that this study was conducted prior to the current Guideline recommendations.

2. Acute neurotoxicity study in rats:

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 45357501), groups of fasted, 42 day old, Alpk:ApfSD (Wistar-derived), rats (10/sex) were given a single oral dose of acetochlor (94.7% a.i., batch/lot # P11) at doses of 0, 150, 500 or 1500 mg/kg bw and observed for 14 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 10 animals/sex/group at pre-test and study Days 1 (time of peak effect), 8, and 15. Cholinesterase activity was not determined. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, 5 rats/sex from the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

Effects of treatment were limited to the highest dose tested (1500 mg/kg bw). Body weights adjusted for initial weight were significantly lower than the control group on Day 8 for males and on Days 1 (peak effect), 8, and 15 for females. Body weight gains were significantly lower for the Day –7 to 8 time period for males (77% of controls) and during throughout the study for females (65-76% of controls. Food consumption by the high-dose males and females was significantly reduced during the first week of the study compared with the controls.

During the FOB, findings were limited to the time of peak effect at the high-dose level. These consisted of hunched posture observed in 5-6 animals/sex, piloerection on 7-10/sex, and staining around the mouth seen in 3-4/sex. The severity was considered slight in the males and from slight to moderate in females. Other findings at 1500 mg/kg bw were decreased activity in one female, chromodacryorrhea in one female, hypothermia in one female, labored breathing in one male, sides pinched in in one male, and upward curvature of the spine in one female. No effects of treatment were noted on landing foot splay measurement, time to tail-flick, or grip strengths.

No effects of treatment were noted for motor activity in males. Total activity counts for high-dose females on Day 1 were significantly decreased compared to controls (251.7 vs 571.4 for controls) and were decreased by 43.8% of the pre-test value.

There were no treatment-related effects on brain weights or gross and histologic pathology or neuropathology.

Based on the effects seen in this study, the LOAEL was 1500 mg/kg bw/day based on decreased body weights and body weight gain (males and females),

DATA EVALUATION RECORD

ALACHLOR

STUDY TYPE: 4-WEEK INHALATION TOXICITY – RAT (OCSPP 870.3465)

MRID 49031001

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
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Arlington, VA 22202

Prepared by
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Knoxville, Tennessee 37922

Task Order No. 6-87

Primary Reviewer: Thomas C. Marshall, Ph.D., DABT	Signature: Date:	Thomas C Marshall AE 2/17/2014
Secondary Reviewers: H.T. Borges, Ph.D, MT(ASCP), DABT	Signature: Date:	H.T. Borges AE 2/17/2014
Robert H. Ross, M.S., Program Manager	Signature: Date:	Robert H Ross AE 02/17/2014
Quality Assurance: Jennifer L. Goldberg, B.S.	Signature: Date:	Jennifer L Goldberg AE 02/17/2014

Disclaimer
This review may have been altered subsequent to the contractor's signatures above.

Summitec Corp. for the U.S. Environmental Protection Agency under Contract No.EP-W-11-014

EPA Reviewer: Ayaad Assaad, D.V.M., Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** 12/18/2015
EPA Secondary Reviewer: Abdallah Khasawinah **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** Dec. 8, 2015

Template version 09/11

TXR#: 0056872

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Inhalation Toxicity - Rat
OCSPP 870.3465.

PC CODE: 121601 **DP BARCODE:** D412326

TEST MATERIAL (PURITY): Alachlor (94.3% a.i.)

SYNONYMS: ***2-Chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide***

CITATION: Roloff, M., Dudek, B., Ribelin, W. (1987). Revision of MSL6779: One-month toxicity study of Alachlor administered to male and female Sprague-Dawley rats by inhalation. Monsanto Company Environmental Health Laboratory, St. Louis, MO. Study No. EHL83112, Submitted April 18, 2013. MRID 49031001. Unpublished.

SPONSOR: Monsanto Company, St. Louis, MO.

EXECUTIVE SUMMARY:

In a whole-body inhalation toxicity study (MRID 49031001) Alachlor (Lot No. MDLT 0801 B; 94.3% purity) was administered as an aerosol to male and female Sprague-Dawley rats (15/sex/concentration) for 6 hours/day, 5 days/week for 4 weeks at mean analytical concentrations of 0, 0.06, 0.22, or 0.51 mg/L. The MMADs (mass median aerodynamic diameters) were 2.3, 2.1, and 2.2 µm for the low-, mid- and high concentrations, respectively.

All animals survived until scheduled sacrifice. Redness around the nose and mouth was observed in males and females in the mid and high exposure groups. Red discharge around the nose, mouth, and to some extent near the eyes was observed following each exposure in low incidence at the low concentration, but was evident in all animals at the mid and high concentrations. Salivation was observed in all animals following exposure at the mid and high concentrations throughout the experimental period. Statistically significant lower body weight was observed in males at the high concentration on days 15 and 22, and in females at the mid and high concentration beginning on day 8 or 15 and continued through the end of the experimental period. Lower mean platelet counts were noted in males at each exposure concentration in a dose-related manner, but statistically lower only at the high concentration. Statistical increases in the red blood cell count, hematocrit, and hemoglobin concentration were observed in males at the low and high exposure, but not at the mid exposure concentration. Glucose concentrations were lower in males at the high exposure and in females at the mid and high exposure. Absolute

kidney weight at the mid concentration and kidney relative-to-body weight at the mid and high concentration were statistically higher in males. Kidney relative-to-body weight at the high concentration was statistically higher in females. Absolute and relative liver weights were statistically higher in males at the high exposure concentration. Relative liver and brain weights at the high concentration were statistically higher in females. No test material-related microscopic lesions were observed.

Based on the effects seen in this study, a NOAEL in male and female Sprague-Dawley rats was the aerosol concentration of 0.06 mg/L. A LOAEL in male and female rats was the aerosol concentration of 0.22 mg/L based on clinical signs (redness around the nose, mouth, and to some extent eyes was observed in males and females and salivation) and organ weight changes (liver and kidney). Body weight decreases although statistically significant were less than 6%.

This 4-week subchronic inhalation toxicity study in the rat is **Acceptable (Guideline)** and satisfies the Guideline requirement for a subchronic inhalation study in the rat (OCSPP 870.3465; OECD 413). A list of deficiencies is given at the end of this document, but it is noted that this study was conducted prior to the current Guideline recommendations.

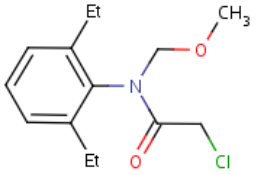
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Alachlor

Description: Cream tan
Lot/batch #: MDLT 0801 B
Purity: 94.3% a.i.
CAS # of TGAI: 15972-60-8
Structure:



2. Vehicle: Filtered air

3. Test animals:

Species: Rat
Strain: Crl:CD(SD)
Age/weight at study initiation: ~8 weeks/ Males 262-298 g; Females 155-194 g
Source: Charles River Ltd., Portage, MI
Housing: Individually in stainless steel wire mesh cages when not in inhalation chambers
Diet: Certified Rodent LabDiet® 5002, Purina Laboratory, *ad libitum* except during exposure.
Water: Reverse osmosis-treated tap water, *ad libitum* except during exposure.
Environmental conditions:
- **Temperature:** 70-74 °F
- **Humidity:** 35-60%
- **Air changes:** Not provided
- **Photoperiod:** 12 hours light/dark

Acclimation period: at least 9 days

B. STUDY DESIGN:

1. In life dates: Start: September 14, 1983; End: October 14, 1983

2. Animal assignment and treatment: Animals were and randomly assigned by weight to the test groups noted in Table 1. No details were provided on the selection of exposure concentrations.

TABLE 1. Study Design				
Experimental parameter	Alachlor Concentrations (mg/L)			
	0	0.06	0.20	0.60
Number of Rats				
Total no. of animals assigned	30 (15/sex)	30 (15/sex)	30 (15/sex)	30 (15/sex)
Sacrifice and necropsy	30 (15/sex)	30 (15/sex)	30 (15/sex)	30 (15/sex)
Measured Concentration				
Achieved aerosol concentration (mg/L ±s.d.)	NA	0.06 ± 0.01	0.22 ± 0.06	0.51 ± 0.19

Data obtained from page 15 (MRID 49031001)

3. **Generation of the test atmosphere / chamber description:** Chamber atmospheres were generated using a spraybar system or syringe pump connected to a vertical particle size separator and coupled with a distribution system that delivered a controlled amount of droplet aerosol and filtered compressed air to the inlet of each 1.75 M^3 Rochester-type stainless steel whole-body exposure chamber. The particle size separator was used to eliminate large droplets and to dilute and conduct the test atmosphere to the top inlet of the chamber. The exposure chambers (one for each exposure level) were fitted with air flow meters and sampling devices to measure particle size and test article concentration.

4. **Test atmosphere concentration:** The exposure concentrations in each exposure chamber were measured by sampling 10 L of each test atmosphere from the animal breathing zone through an impinger containing methanol four times per day. Analysis was conducted by gas chromatography with flame ionization detection. Results are shown in Table 1. Homogeneity of concentration within each chamber was tested twice during the experimental period and resulted in coefficients of variation of 25% and 17 % for the low concentration, 8% and 19% for the mid concentration, and 11% and 10% for the high concentration. Time to equilibrium was not reported.

5. **Particle size determination:** Particle size for each exposure concentration was determined at least seven or eight times during the study using an Andersen impactor (Andersen Samplers Inc., Atlanta, GA). The mass median aerodynamic diameter (MMAD) and geometric standard deviation (σg) were 2.3 μm (2.1), 2.1 μm (1.9), and 2.2 μm (1.8) for the low, mid, and high concentration groups, respectively.

6. **Statistics:** All analyses were conducted comparing each test substance-exposed group to the control group by sex. The overall level of significance for intergroup differences was $p \leq 0.05$. Body weights and organ weights were subjected to a one-way parametric analysis of variance (ANOVA) to determine intergroup differences. When significant differences were detected, Dunnett's test was used to compare the test substance-exposed groups to the control group. Noncategorical clinical pathology data were analyzed by Dunnett's test. Organ-to-body-weight ratios were analyzed using the Mann-Whitney test with the Bonferroni Inequality Modification Procedure. Incidence data on microscopic lesions were analyzed using the Fisher exact test with the Bonferroni Inequality Modification Procedure for comparing unequal groups. Statistical methods for analyzing urinalysis data and categorical clinical pathology data were not described.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were observed before, once during, and after each exposure, as well as twice daily on non-exposure days for clinical signs, abnormal behavior, morbidity, and mortality.

1b. Clinical examinations: Detailed clinical examinations were conducted weekly.

1c. Neurological evaluations: Neurological evaluations were not conducted.

2. Body weight: Animals were weighed weekly during the experimental exposure period and at study termination prior to necropsy.

3. Food consumption: Food consumption was not recorded.

4. Ophthalmoscopic examination: Eyes of all rats were examined during week 4.

5. Hematology and clinical chemistry: Blood was collected from all surviving animals at termination for hematology and clinical chemistry; the animals were fasted overnight prior to blood collection. Blood was collected from the posterior vena cava while under chloroform anesthesia. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		Red cell morphology
	(Thromboplastin time)		Hemoglobin distribution width
	(Clotting time)		RBC distribution width
	(Prothrombin time)		Mean platelet volume

* Recommended for subchronic inhalation studies based on Guideline 870.3465

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
	Calcium	X	Albumin*
	Chloride		Creatinine*
	Magnesium	X	Urea nitrogen*
	Phosphorus		Total Cholesterol*
	Potassium*	X	Globulins
	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase*	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin/Globulin ratio
X	Alanine aminotransferase (ALT/also SGPT)*		Appearance
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

c. Cholinesterase determination: Not conducted.

6. Urinalysis: Urine was collected from all animals after overnight fasting at study termination. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
	Sediment (microscopic)		Nitrites
X	Protein*	X	Urobilinogen

* Optional for inhalation toxicity studies

7. Sacrifice and pathology: Rats were killed by exsanguination while under chloroform anesthesia. Any gross lesions were recorded and tissues were collected and preserved from animals in all exposure groups. Tissues from the control and high concentration groups were processed and subjected to microscopic evaluation. The CHECKED (X) tissues were collected and the (XX) organs were weighed.

X	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
	Duodenum*
	Jejunum*
X	Ileum*
	Cecum*
X	Colon*
	Rectum*
XX	Liver*+
	Gall bladder* (not rat)
	Bile duct* (rat)
X	Pancreas*

X	RESPIRATORY
X	Trachea*
X	Lung*
X	Nose*
	Pharynx*
	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta, thoracic*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
X	Thymus*+

X	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
	Seminal vesicles*
X	Ovaries*+
X	Uterus*+
X	Mammary gland*
	Cervix
	Oviduct
	Vagina

X	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve*
X	Spinal cord*
X	Pituitary*
X	Eyes (optic nerve)*

X	GLANDULAR
X	Adrenal gland*+
	Lacrimal gland
X	Parathyroid*
X	Thyroid*

X	OTHER
X	Bone (sternum and/or femur)
X	Skeletal muscle
X	Skin
X	All gross lesions and masses*
	Blood smear
	Harderian gland
	Carina
	Peyer's patches

* Recommended for subchronic rodent studies based on Guideline 870.3465
+ Organ weights required

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs and Mortality:** All animals survived until scheduled sacrifice. Redness around the nose and mouth was observed in males and females in the mid and high exposure groups. Red discharge around the nose, mouth, and to some extent near the eyes was observed following each exposure in low incidence at the low concentration, but was evident in all animals at the mid and high concentrations. Salivation was observed in all animals following exposure at the mid and high concentrations throughout the experimental period, while hypoactivity was observed in 3/15 males and one female at the mid concentration and in three males and eleven females at the high concentration during the first week of exposures.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Statistically significant lower body weight was observed in males at the high concentration on days 15 (↓5%, $p<0.01$) and 22 (↓5%, $p<0.05$), and in females at the mid (↓5 – 6%) and high (↓4 – 10%) concentration ($p<0.05$ or $p<0.01$) beginning on day 8 or 15 and continued through the end of the experimental period (Table 2). Weight gain was 16% lower in high concentration males by the end of the experimental period, and 20% and 33% lower in females at the mid- and high concentrations, respectively.

TABLE 2. Mean body weight and body weight gain during 4 weeks of inhalation exposure to alachlor[a]

Analytical concentration (mg/L)	Body weights (g ±SD)					Total weight gain	
	Day 1	Day 8	Day 15	Day 22	Day 28	g	% from control[b]
Male							
0	277 ± 9.1	306 ± 11.5	339 ± 14.3	364 ± 13.9	384 ± 17.4	+107	NA
0.06	277 ± 10.	308 ± 15.4	338 ± 17.7	366 ± 21.3	387 ± 21.1	+110	+3
0.22	279 ± 10.2	299 ± 10.9	328 ± 12.8	356 ± 14.8	376 ± 17.2	+97	-9
0.51	278 ± 9.7	297 ± 11.7	322** ± 14.6 (↓5%)	346* ± 17.9 (↓5%)	368 ± 19.7	+90	-16
Female							
0	182 ± 7.7	201 ± 10.2	220 ± 9.8	235 ± 10.8	242 ± 12.8	+60	NA
0.06	183 ± 9.1	198 ± 7.1	214 ± 9.7	228 ± 9.8	236 ± 10.7	+53	-12
0.22	183 ± 6.7	194 ± 7.1	209** ± 7.3 (↓5%)	221** ± 9.7 (↓6%)	231* ± 9.1 (↓5%)	+48	-20
0.51	181 ± 6.2	193* ± 7.7 (↓4%)	205** ± 10.5 (↓7%)	211** ± 13.4 (↓10%)	221** ± 12.0 (↓9%)	+40	-33

[a] Data obtained from pages 27-28 of MRID 49031001.
[b] Calculated by reviewer.

C. FOOD CONSUMPTION: Food consumption was not monitored.

D. BLOOD ANALYSES:

1. **Hematology:** Lower mean platelet counts were noted in males at each exposure concentration in a dose-related manner, but statistically lower ($p<0.01$) only at the high concentration. Statistical increases ($p<0.05$ or $p<0.01$) in the red blood cell count, hematocrit, and hemoglobin concentration were observed in males at the low and high exposure, but not at the mid exposure concentration. The investigators stated that the statistical differences observed in the hematology values were within the historical control ranges (historical data not provided).

2. **Clinical chemistry:** Glucose concentrations were ~30% lower in males ($p<0.05$) at the high exposure and in females ($p<0.01$) at the mid and high exposure. The changes in glucose levels between males and females was not considered to be biologically significate since it depends on each individual animal and it's level of activity during exposure (historical data not provided).

E. URINALYSIS: No treatment-related effects on urinalysis parameters were observed.

F. OPHTHALMOSCOPIC EXAMINATION: No lesions were observed during the ophthalmoscopic examinations.

G. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** Dilation of the uterine lumen was observed in both control and treated groups and was not considered treatment related. No other findings were observed.

2. **Organ weight:** Selected organ weight data are presented in Table 3. Absolute kidney weight at the mid concentration (↑8%) and kidney relative-to-body weight at the mid (↑11%) and high (↑12%) concentration were statistically higher in males ($p<0.05$). Absolute heart weight, but not relative-to-body weight, was statistically lower in females at the mid (↓10%, $p<0.01$) and high (↓8%, $p<0.05$) concentrations. Kidney relative-to-body weight at the high concentration was statistically higher in females (↑11%, $p<0.05$). Absolute (↑9%) and relative (↑15%) liver weights were statistically higher in males at the high exposure concentration ($p<0.05$). Relative liver (↑15%) and brain (↑8%) weights at the high concentration were statistically higher in females ($p<0.05$). No microscopic correlates were observed for these findings.

Table 3. Changes in selected organ weights following 4 weeks of inhalation exposure. to alachlor. [a]

Organ	Analytical exposure concentration (mg/L)			
	0	0.06	0.22	0.51
MALES				
Absolute weight (g)				
Brain	1.975 ± 0.024	1.984 ± 0.022	1.949 ± 0.022	1.957 ± 0.023
Heart	1.210 ± 0.018	1.266 ± 0.037	1.180 ± 0.026	1.185 ± 0.024
Kidney	2.669 ± 0.063	2.834 ± 0.056	2.890* ± 0.061 (↑8%)	2.835 ± 0.050
Liver	11.386 ± 0.265	11.843 ± 0.308	11.753 ± 0.269	12.429* ± 0.217 (↑9%)
Relative to body weight (g/100g body weight)				
Brain	0.562 ± 0.007	0.563 ± 0.008	0.566 ± 0.009	0.586 ± 0.008
Heart	0.345 ± 0.007	0.358 ± 0.007	0.343 ± 0.009	0.355 ± 0.007
Kidney	0.759 ± 0.017	0.804 ± 0.016	0.839* ± 0.019 (↑11%)	0.849* ± 0.019 (↑12%)
Liver	3.23 ± 0.071	3.358 ± 0.085	3.408 ± 0.075	3.718* ± 0.057 (↑15%)
FEMALES				
Absolute weight (g)				
Brain	1.863 ± 0.020	1.858 ± 0.024	1.825 ± 0.019	1.823 ± 0.017
Heart	0.871 ± 0.023	0.827 ± 0.018	0.780** ± 0.013 (↓10%)	0.804* ± 0.016 (↓8%)
Kidney	1.695 ± 0.043	1.743 ± 0.030	1.649 ± 0.029	1.701 ± 0.045
Liver	7.133 ± 0.207	7.572 ± 8.256	7.038 ± 0.129	7.351 ± 0.218
Relative to body weight (g/100g body weight)				
Brain	0.859 ± 0.011	0.865 ± 0.016	0.882 ± 0.010	0.932* ± 0.013 (↑8%)
Heart	0.401 ± 0.011	0.385 ± 0.008	0.377 ± 0.006	0.410 ± 0.006
Kidney	0.781 ± 0.020	0.811 ± 0.012	0.797 ± 0.015	0.868* ± 0.021 (↑11%)
Liver	3.278 ± 0.071	3.516 ± 0.096	3.402 ± 0.065	3.758* ± 0.114 (↑15%)

Data obtained from pages 105-117 of MRID 49031001.
Values expressed as group means ± s.e.
*p<0.05; **p<0.01

3. **Microscopic pathology:** The type and incidence of microscopic findings were similar for the control and high concentration animals. No test material-related microscopic lesions were observed.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Alachlor, as administered in this study, caused no deaths, but resulted in clinical signs related to sensory irritation, depressed body weights, and lower glucose concentrations in rats at the mid- and/or high exposure concentrations. The investigators concluded that the no-observed-adverse-effect level (NOAEL) for both males and females was 0.06 mg/L.

B. REVIEWER COMMENTS: Redness around the nose, mouth, and to some extent eyes was observed in males and females in the mid- and high exposure groups. Salivation was observed in all animals following exposure at the mid and high concentrations throughout the experimental period. These clinical signs were indicative of sensory irritation. Statistically significant lower body weight was observed in males at the high concentration and in females at the mid- and high concentration. Hematology differences observed in platelet count, red blood cell count, hematocrit, and hemoglobin concentration were stated by the investigators to be within historical control ranges, but the historical data were not provided. Other deficiencies are listed below. It is noted that this study was conducted prior to publication of the current Guideline 870.3465.

Based on the effects seen in this study, a NOAEL in male and female Sprague-Dawley rats was the aerosol concentration of 0.06 mg/L. A LOAEL in male and female rats was the aerosol concentration of 0.22 mg/L based on clinical signs (redness around the nose, mouth, and to some extent eyes was observed in males and females and salivation) and organ weight changes (liver and kidney). Body weight decreases although statistically significant were less than 6%.

This 4-week subchronic inhalation toxicity study in the rat is **Acceptable (Guideline)** and satisfies the Guideline requirement for a subchronic inhalation study in the rat (OCSPP 870.3465; OECD 413). A list of deficiencies is given at the end of this document, but it is noted that this study was conducted prior to the current Guideline.

C. STUDY DEFICIENCIES:

- The following hematology and clinical chemistry endpoints recommended for subchronic inhalation studies (Guideline 870.3465) were not conducted (minor):
  - Blood clotting measurements (thromboplastin time, clotting time, prothrombin time).
  - Potassium, sodium, creatinine, total cholesterol.
- The following organ weights recommended for subchronic inhalation studies (Guideline 870.3465) were not obtained: Thymus, ovaries, uterus, adrenal gland (minor).
- The following tissues were not obtained for microscopic examination as recommended for subchronic inhalation studies (Guideline 870.3465): duodenum, jejunum, rectum, pharynx, larynx, seminal vesicles (minor).
- Hematology differences observed in platelet count, red blood cell count, hematocrit, and hemoglobin concentration were stated by the investigators to be within historical control ranges, but the historical data were not provided (minor).
- The time to equilibrium in the exposure chamber was not reported (minor).

DATA EVALUATION RECORD

ACETOCHLOR/121601
[OPPTS §870.6200A]

STUDY TYPE: ACUTE NEUROTOXICITY - RAT
MRID 45357501

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-50

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Template version 11/01

DATA EVALUATION RECORD
TXR#: 0057234

STUDY TYPE: Acute Neurotoxicity - Rats [OPPTS 870.6200a (§81-8)] OECD 424.

PC CODE: 121601 **DP BARCODE:** D274337
SUBMISSION NO.: S595593

TEST MATERIAL (PURITY): Acetochlor (94.7% a.i.)

SYNONYMS: 2-Chloro-*N*-ethoxymethyl-6'-ethylacet-*o*-toluidide

CITATION: Kilgour, J.D. (2001) Acetochlor: Acute Neurotoxicity Study in Rats. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK. Study Number: CTL/AR6884. February 8, 2001. MRID 45357501. Unpublished

SPONSOR: Acetochlor Registration Partnership, c/o Monsanto Company, St. Louis, MO

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 45357501), groups of fasted, 42 day old, Alpk:ApfSD (Wistar-derived), rats (10/sex) were given a single oral dose of acetochlor (94.7% a.i., batch/lot # P11) at doses of 0, 150, 500 or 1500 mg/kg bw and observed for 14 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 10 animals/sex/group at pre-test and study Days 1 (time of peak effect), 8, and 15. Cholinesterase activity was not determined. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, 5 rats/sex from the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

Effects of treatment were limited to the highest dose tested (1500 mg/kg bw). Body weights adjusted for initial weight were significantly lower than the control group on Day 8 for males and on Days 1 (peak effect), 8, and 15 for females. Body weight gains were significantly lower for the Day –7 to 8 time period for males (77% of controls) and

during throughout the study for females (65-76% of controls. Food consumption by the high-dose males and females was significantly reduced during the first week of the study compared with the controls.

During the FOB, findings were limited to the time of peak effect at the high-dose level. These consisted of hunched posture observed in 5-6 animals/sex, piloerection on 7-10/sex, and staining around the mouth seen in 3-4/sex. The severity was considered slight in the males and from slight to moderate in females. Other findings at 1500 mg/kg bw were decreased activity in one female, chromodacryorrhea in one female, hypothermia in one female, labored breathing in one male, sides pinched in in one male, and upward curvature of the spine in one female. No effects of treatment were noted on landing foot splay measurement, time to tail-flick, or grip strengths.

No effects of treatment were noted for motor activity in males. Total activity counts for high-dose females on Day 1 were significantly decreased compared to controls (251.7 vs 571.4 for controls) and were decreased by 43.8% of the pre-test value.

There were no treatment-related effects on brain weights or gross and histologic pathology or neuropathology.

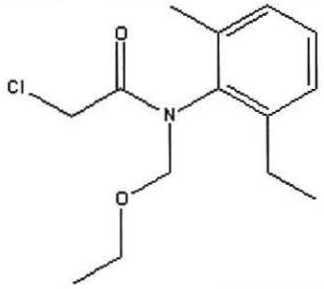
Based on the effects seen in this study, the LOAEL was 1500 mg/kg bw/day based on decreased body weights and body weight gain (males and females), reduced food consumption (males and females), increased incidence of clinical signs during the FOB, and decreased motor activity in females at the time of peak effect, with a NOAEL of 500 mg/kg bw/day.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:		Acetochlor
	Description:	light brown liquid, analyzed April 2000 and every 6 months thereafter
	Lot/Batch #:	P11
	Purity:	94.7 % a.i.
	CAS # of TGAI:	34256-82-1
	Structure:	

2. **Vehicle and/or positive control:** The vehicle used was Mazola corn oil (CTL test substance reference number Y00790/007). There was no positive control used in this study.

3. Test animals:			
	Species:	Rat	
	Strain:	Alpk:APfSD (Wistar-derived)	
	Age/weight at dosing:	At least 42 days; males, 163-205 g; females, 127-161	
	Source:	Rodent Breeding Unit, Alderley Park, Macclesfield Cheshire, UK	
	Housing:	5/cage by sex in "multiple rat racks suitable for animals of this strain and the weight range expected during the course of this study"	
	Diet:	CT1 diet, Special Diet Services Limited, Stepfield, Witham, Essex, UK, *ad libitum*	
	Water:	Mains water supplied by an automatic system, *ad libitum*	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22∀3EC 30-70% at least 15/hr 12 hrs dark/ 12 hrs light
	Acclimation period:	At least 13 days	

B. STUDY DESIGN:

1. **In life dates:** Start: January 19, 2000; End: May 3, 2000

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1 by a procedure described in Appendix C of the report. Any animals that failed to respond to a randomized tail-flick test, had adverse clinical signs and/or were at extremes in the weight range were excluded from the study. Remaining animals were randomized to groups using a Latin Square that was generated using

animal weight. Sexes were randomized separately. There were four replicates in a randomized-block design, which contained one cage per treatment group. Administration was staggered over a 4-day interval to facilitate neurobehavioral observations. Males (replicates 1 and 3) and females (replicates 2 and 4) were treated on separate days. Following an overnight fast, rats were given a single dose by gavage in Mazola corn oil in a dosing volume of 10 ml/kg. Rats were observed daily and were weighed on Day –7, prior to dosing on Day 1, at ~4-5 hours after dosing, and on Days 8 and 15. Survivors were sacrificed and a necropsy was not performed. The one male that died on study was subjected to gross examination. The five animals/sex/group designated for neuropathology were subjected to an external examination only.

Dose levels were selected based on the results of a preliminary range-finding study using dose levels of 0, 1500 or 2000 mg/kg bw of acetochlor which was administered in a single dose by gavage to 3 Alpk:ApfSD rats/sex/group. Clinical observations were recorded and a limited FOB was performed at approximately hourly intervals for 8 hours post dosing and daily thereafter. Individual body weights were recorded on day –1, immediately prior to dosing on day 1, and at the same time each FOB was performed. The time to peak effect was determined to be 4-5 hours for both dose levels and both sexes. Clinical signs observed during the 8 hours after dosing for males and females administered the high dose included lacrimation, salivation, piloerection, hunched posture, stains around the nose and mouth, and diarrhea. No signs were observed on Day 2. For females recovery did not occur until Day 7. The signs observed after Day 1 included decreased activity, irregular breathing, chromodacryorrhea, hunched posture, piloerection, and ptosis. Clinical signs were also observed at 1500 mg/kg bw which consisted of decreased activity and signs of salivation, stains around the nose, signs of diarrhea, urine stains, and chromodacryorrhea. Signs were absent by Day 4 in males and Day 8 in females. Body weights were decreased in both males and females at both dose levels, but did not reach statistical significance. Body weight gain was statistically decreased in males and females at 2000 mg/kg bw and for females only at 1500 mg/kg bw.

TABLE 1. Study design

Experimental parameter	Dose group (mg/kg bw)			
	0	150	500	1500
Total number of Animals/sex/group	10	10	10	10
Behavioral Testing (FOB, Motor Activity)	10	10	10	10
Neuropathology	5	5	5	5

3. **Test substance preparation and analysis**: An appropriate amount of corn oil was added to a weighed amount of test material (amount adjusted for purity). One preparation was made for each dose group. The four dose preparations were divided

into four replicates for each day of dosing and were stored in the dark at room temperature for up to four days. Prior to study initiation, each dose solution was analyzed to verify the achieved concentrations of the test material in corn oil. Homogeneity was determined prior to dosing in the low- and high-dose solutions. Stability of acetochlor in corn oil was determined for the low-, mid-, and high-dose solutions for up to 11 days.

Results:

Homogeneity analysis: The values for the 15 mg/mL solution ranged from 14.8 to.15.1, mean 15.0 mg/mL, for the top, middle, and bottom of the test material in corn oil. The values for the 150 mg/mL solution ranged from 149 to 151 mg/mL; mean 150 mg/mL.

Stability analysis: The achieved concentration for the low-dose solution was 100.0% of nominal on day 0, 100.7% of nominal on day 7, and 104.9% of nominal on day 11. The achieved concentration for the mid-dose solution was 100.0% of nominal on day 0, 102.7% of nominal on day 7, and 104.4% of nominal on day 11. The achieved concentration for the high-dose solution was 100.0% of nominal on day 0, 103.4% of nominal on day 7, and 100.7% of nominal on day 11.
Concentration analysis: control—not detectable
Low dose—100.0% of nominal; range 14.9 to 15.1 mg/mL; mean 15.0 mg/mL
Mid dose—100.6% of nominal; range 50.1 to 50.4 mg/mL; mean 50.3 mg/mL
High dose—100.0% of nominal; range 150 to 151 mg/mL; mean 150 mg/mL

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics: According to page 23 of the report, "[a]ll data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the MIXED procedure in SAS" (SAS Institute Inc. SAS/STAT Software: Changes and Enhancements through release 6.11, Cary, NC., SAS Institute Inc., 1966). Appendix E of the report described the statistical methods used as follows: Body weights were considered by analysis of covariance on initial (day –7) bodyweight, separately for males and females.

Weekly food consumption, time to tail-flick (in the sensory function test), landing foot splay and grip strengths were considered by analysis of variance, separately for males and females.

Total motor activity was considered by a repeated measures analysis of variance, separately for males and females. The pattern of motor activity habituation was analyzed by visual inspection of the data.

Brain weight was considered by analysis of variance and analysis of covariance on final body weight, separately for males and females. Summary data are presented for organ-to-body weight ratios but these were not analyzed statistically as the analysis of covariance provides a better method of allowing for differences in terminal body weights.

With the exception of motor activity, analyses of variance and covariance allowed for the replicate structure of the study design and were carried out using the MIXED procedure in SAS. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least –squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

C. METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed daily for mortality and morbidity. Either detailed clinical observations or detailed FOB clinical observations were recorded daily.

2. **Body weight:** Animals were weighed on day –7, prior to dosing on day 1, at approximately 4-5 hours after dosing (time of peak effect) on day 1, and on days 8 and 15.

3. **Food consumption:** Food consumption was recorded throughout the study for each cage of rats. It was calculated at weekly intervals and presented as a mean value of g food/rat/day for each cage.

4. **Cholinesterase determination:** Not applicable. Cholinesterase activity was not measured.

5. **Neurobehavioral assessment:**

a. **Functional Observational Battery (FOB):** All observations and quantitative measures were performed on Day –7, Day 1 (time of peak effect), Day 8, and Day 15. For detailed clinical observations, each animal was removed from the cage and physically examined for changes in general health status and quantitative assessments. Observations were made by one technician who was 'blind' with respect to treatment group. Observations were recorded directly into a computer system by personnel not involved in describing clinical observations. The degree of findings was scored as slight, moderate or extreme as appropriate. Righting reflex was determined by placing the rat in a supine position; response to sound by finger click/clack; splay reflex by degree of splay when the animal was lifted by the base

of the tail; visual placing response by lifting the animal by the base of the tail and slowly moving it downwards towards the edge of the arena; pupil response to light after the eye had been held closed for 10 seconds; palpebral membrane reflex by touching the membrane with a bristle and observing the blink response; corneal reflex by toughing a hair against the cornea and observing the blink reflex (only performed if the palpebral reflex was absent); and foot withdrawal reflex by response to toe pinch. The methods used for quantitative measures (forelimb and hind-limb grip strength, landing foot splay, and time to tail flick) were not described and the length of time each animal was in the standard arena was not provided.

The CHECKED (X) parameters were examined:

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
	Posture*		Reactivity*	X	Mobility
X	Bizarre behavior	X	Lacrimation* / chromodacryorrhea		Rearing+
	Convulsions*	X	Salivation*	X	Arousal/ gereral activity level*
	Tremors*	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
	Palpebral closure*		Palpebral closure*	X	Abnormal movements*
X	Volcalization	X	Respiratory rate+	X	Urination / defecation*
		X	Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*		Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response*	X	Tremors	X	Backing
X	Pain response*	X	Convulsions	X	Reduced limb function
X	Pupil response*	X	Hypothermia/hyperthermia	X	Piloerection
X	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension				
	Hindlimb extension	X	Body weight*		Hindlimb extensor strength
X	Air righting reflex+		Body temperature+	X	Forelimb grip strength*
	Olfactory orientation			X	Hindlimb grip strength*
X	Palpebral membrane reflex/corneal reflex			X	Landing foot splay*
X	Pinna reflex		**OTHER OBSERVATIONS**		Rotarod performance
X	Foot withdrawal reflex		Observations that might facilitate interpretation of the data were recorded	X	Time to tail flick

*Required parameters; +Recommended parameters

b. Locomotor activity: Locomotor activity was evaluated on the following study days: Day –7, Day 1 at time of peak effect, Day 8, and Day 15. The time of the measurements in relation to the time of the FOBs was not provided. A Coulbourn Lab Linc Infra-red Motion Activity System that records small and large movements as an activity count was used. Each animal was placed individually into the apparatus (activity monitor) that was kept in a separate room to minimize

disturbances, and the session was started immediately. Each session was divided into ten scans of five minutes duration. While activity was being measured, the animals had no access to food or water. When the trials were repeated, each animal was returned to the same activity monitor at approximately the same time of day. Treatment groups were counter-balanced across test times and across devices. From the information provided in Appendix D (page 92 of the report), 20 activity monitors were used. The allocation of rats (by individual animal numbers) to each monitor is tabulated in Appendix D.

6. **Sacrifice and pathology:** At study termination (Day 15), 5 rats/sex were anesthetized and killed by perfusion fixation with modified Karnovsky's fixative. The tissues from these animals were submitted for neuropathology. The remaining animals were killed by overexposure to the halothane Ph. Eur. Vapor, exsanguinated, and discarded. Brain weights were recorded for the 5 rats/sex that were perfused and these same animals were subjected to an external examination only.

   Tissues were processed as follows: Transverse sections of the brain, gastrocnemius muscle, eye, spinal cord, dorsal root ganglia, and spinal nerve roots were trimmed and embedded in paraffin wax. Longitudinal sections of spinal cord were trimmed and embedded in paraffin wax. From these blocks 5 um sections were cut and stained with hematoxylin and eosin. Transverse and longitudinal sections of proximal sciatic nerve, proximal tibial nerve, and distal tibial nerve (tibial nerve calf muscle branches) were embedded in resin and semi-thin sections cut and stained with toluidine blue. Tissues were examined from animals in the control and high dose groups by light microscopy.

The CHECKED (X) tissues were evaluated.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	9 levels	X	Proximal sciatic nerve
	Forebrain		Mid-thigh
	Center of cerebrum		Sciatic Notch
	Midbrain		
	Cerebellum		**OTHER**
	Pons	X	Sural Nerve
	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian Ganglion	X	Cervical dorsal root fibers
	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve		
X	Eyes		
X	Gastrocnemius muscle		

7. Positive controls: Positive control data were provided to validate the ability of the procedures and observers of the performing lab to detect the effect of chemicals on FOB parameters, motor activity, behavior, neuropathological lesions, and other parameters indicative of neurotoxicity. These data were obtained from MRIDs #45811002 & 45811003 (December 2000). **Amphetamine sulfate** (10 mg/kg bw; single i.p. dose; MRID 45811002) induced the following in both sexes: increased activity, piloerection, salivation, and urinary incontinence; and decreased forelimb grip strength. Decreased hindlimb grip strength was also observed in males. **Morphine sulphate** (100 mg/kg bw; single gavage dose; MRID 45811003) induced increased time to tail flick in both sexes and decreased forelimb grip strength in females.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** Clinical signs of toxicity were only observed in the one male rat in the high dose group that died on study. Signs consisted of hunched posture, stains around the mouth, dry urine stains, subdued, stains around the nose, and thin appearance. The death was considered to be treatment related by the study authors. Clinical observations noted during the FOB are described under Neurobehavioral Results below.

2. **Mortality:** There was one death during the study, male (#137) in the high dose group that was found dead on Day 2 of the study. This death was attributed to treatment.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** Selected data are given in Table 2. For males in the high-dose group, body weights adjusted for initial body weight were statistically significantly lower than the control group on Day 8 and body weight gain was significantly lower for the Day –7 to 8 time period. Unadjusted body weights were used for determining body weight gain for each time period. For females in the high dose group, body weight adjusted for initial body weight was statistically significantly lower than the control group on Day 1 at the time of peak effect, on Day 8, and on Day 15. Body weight gain was significantly lower during the following time periods: Day –7 to Day 1 at the time of peak effect, Day –7 to 8, and Day –7 to 15.

TABLE 2. Body weight and body weight gain (g)

Observation	0 mg/kg	150 mg/kg	500 mg/kg	1500 mg/kg
Body weight - Males n=	**10**	**10**	**10**	**10[a]**
Day 1	184.7 ± 13.0	186.4 ± 12.3	181.7 ± 10.3	183.1 ± 11.1
Day 1peak effect (adjusted)	198.5 ± 15.2 (198.3)	199.8 ± 12.1 (199.4)	197.4 ± 11.3 (197.6)	193.1 ± 13.1 (193.5)
Day 8 (adjusted)	261.8 ± 18.6 (261.3)	262.8 ± 17.7 (262.0)	256.8 ± 13.7 (256.8)	239.1 ± 12.4 (240.8**)
Day 15 (adjusted)	297.6 ± 23.0 (297.1)	303.8 ± 21.4 (303.0)	293.0 ± 17.8 (293.0)	284.0 ± 13.9 (285.5)
Body weight - Females n=	**10**	**10**	**9[b]**	**10**
Day 1	143.2 ± 7.5	139.9 ± 9.3	139.6 ± 8.9	141.6 ± 9.5
Day 1peak effect (adjusted)	155.1 ± 9.4 (154.9)	152.2 ± 11.2 (152.6)	149.4 ± 11.2 (150.8)	150.2 ± 10.8 (148.8*)
Day 8 (adjusted)	183.3 ± 6.6 (183.1)	182.4 ± 10.5 (182.8)	182.4 ± 12.3 (182.7)	171.7 ± 14.0 (170.3**)
Day 15 (adjusted)	200.4 ± 8.5 (200.2)	199.5 ± 11.2 (199.8)	194.2 ± 10.0 (195.3)	186.5 ± 16.3 (185.3**)
Body weight gain - Males n=	**10**	**10**	**10**	**10[a]**
Day -7 to 1	28.6 ± 6.3	29.6 ± 4.2	27.9 ± 4.9	23.8 ± 9.8
Day -7 to 8	91.9 ± 11.0	92.6 ± 9.4	87.3 ± 9.5	71.0** ± 5.9
Day -7 to 15	127.7 ± 18.4	133.6 ± 14.4	123.5 ± 16.6	115.9 ± 8.3
Body weight gain - Females n=	**10**	**10**	**9[b]**	**10**
Day -7 to 1	17.2 ± 4.1	14.9 ± 5.5	13.2 ± 6.8	11.1* ± 3.0
Day -7 to 8	45.4 ± 4.6	45.1 ± 7.1	45.2 ± 6.6	32.6** ± 6.8
Day -7 to 15	62.5 ± 6.5	62.2 ± 10.7	58.0 ± 9.4	47.4** ± 10.7

Data extracted from pages 38 and 40, MRID 45357501.
[a]Reduced to 9 after Day 2 when one male rat died.
[b]Body weight data for female number 166 was apparently not used in calculating the mean body weights and body weight gains in the 500 mg/kg bw group because the Day-7 body weight appears to have been incorrectly recorded (page 108 of the report).
Values represent mean ± S.D
n=number of animals/group
*=p<.05, **=p<.01, when compared to control means.

C. **FOOD CONSUMPTION**: For male rats, food consumption was statistically significantly lower in the 500 and 1500 mg/kg bw groups during the first week of the study (Table 3). For females, food consumption was significantly lower only at 1500 mg/kg bw during week 1. Food consumption was comparable among all groups during week 2 of the study.

TABLE 3. Food consumption (g/kg/day)				
Week	**0 mg/kg**	**150 mg/kg**	**500 mg/kg**	**1500 mg/kg**
Males n=	**2**	**2**	**2**	**2**
Week 1	30.4± 1.1	29.7± 1.5	28.4*± 0.3	23.4**± 0.9
Week 2	31.2± 1.4	31.4± 2.2	29.7± 0.2	30.7± 0.0
Females n=	**2**	**2**	**2**	**2**
Week 1	22.4 ± 0.7	22.0 ± 0.4	22.0 ± 0.1	18.6** ± 1.4
Week 2	20.8 ± 0.8	20.5 ± 0.9	21.2 ± 0.5	20.1 ± 2.2

Data were extracted from page 4, MRID 45357501.
Values represent mean ± S.D.
n=number of cages/group
*=p<.05, **=p<.01, when compared to control means

D. CHOLINESTERASE ACTIVITIES: Not applicable

E. NEUROBEHAVIORAL RESULTS

1. **FOB findings:** Findings considered to be treatment related were limited to the time of peak effect at the high-dose level. In high-dose males and females, these consisted of hunched posture observed in 5-6 animals/sex, piloerection on 7-10/sex, and staining around the mouth seen in 3-4/sex. The severity was considered slight in the males and from slight to moderate in females. Other findings at 1500 mg/kg bw were decreased activity in one female, chromodacryorrhea in one female, hypothermia in one female, labored breathing in one male, sides pinched in in one male, and upward curvature of the spine in one female. Although difficult to conclusively determine, these findings could be related to treatment because some were observed in the preliminary range-finding study.

   No effects of treatment were noted on landing foot splay measurement and time to tail-flick. These data are summarized on pages 67 and 68 of the study report.

   Hind-limb grip strength was statistically significantly decreased in females in the 1500 mg/kg bw on Day 15 only (365∀85 g vs 465∀68 g for controls; p # 0.05). No other effects on hind-limb or forelimb grip strength were observed in treated females nor were any effects observed in the treated males. Historical control data provided for hind-limb grip strength in Appendix G (page 95 of the study report) indicate that the decrease in the high-dose females on Day 15 was within the normal range (200-925 g in 10 studies conducted 1994-2000). The effect on hind-limb grip strength is considered incidental and not treatment related because it only occurred on Day 15, not at the time peak effect, and was within the normal range.

2. **Motor activity:** Total activity counts are given in Table 6. No effects of treatment were noted on total activity counts for males; however, in females total activity counts were statistically significantly decreased on Day 1 in the mid- and high-dose groups and were statistically significantly increased on Day 8 at the high dose compared with the control levels. The effect at 1500 mg/kg bw on Day 1 is considered to be treatment related because the activity was decreased by 43.8% of the pre-test value. The percent of pre-test values for the other groups ranged from 134.9 to 116.3%. The Day 1 effect at 500 mg/kg bw represented a slightly greater value (116.3%) than the pre-test value and therefore, is not considered to be related to treatment. Likewise, the increased total activity counts at the high dose on Day 8 are not considered related to treatment because the effect is not dose-related when compared to the pretest values (124.1% control; 145.4% low-dose group; 165.8% mid-dose group; and 150.2% high-dose group). In addition, acetochlor treatment had no effect on the pattern of habituation in either males or females.

TABLE 4. Motor activity (total activity counts for session)

Test Day	0 mg/kg	150 mg/kg	500 mg/kg	1500 mg/kg
Males (n=10)				
Pre-test (Day –7)	398.5 ± 190.8	390.3 ± 107.4	438.9 ± 133.8	437.6 ± 93.0
Day 1	361.2 ± 234.9	300.5 ± 112.5	388.8 ± 169.7	269.2 ± 122.6
Day 8	566.6 ± 117.6	581.6 ± 144.5	487.0 ± 103.4	575.6 ± 131.8
Day 15	516.3 ± 163.2	538.1 ± 117.8	609.4 ± 86.9	504.2 ± 129.3
Females (n=10)				
Pre-test (Day –7)	423.6 ± 115.9	392.7 ± 130.8	345.5 ± 77.3	448.1 ± 153.9
Day 1	571.4 ± 134.9	501.4 ± 147.4	401.7** ± 176.7	251.7** ± 72.3
Day 8	525.8 ± 134.1	570.9 ± 167.0	573.0 ± 174.4	673.2* ± 122.3
Day 15	622.6 ± 123.0	587.3 ± 135.9	632.3 ± 152.8	642.2 ± 145.3

Data were extracted from pages 71 and 72, MRID 75357501.
Values represent mean ± S.D..
*=p<.05,** p<.01 compared with controls

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** Gross pathology findings were only observed in the high-dose male animal (#137) that died on study. Findings consisted of stained nares and distended stomach with thin wall. There were no macroscopic findings in any other animal.

2. **Brain weight:** No treatment-related effects on brain weights were observed (Table 5).

TABLE 5: Absolute and relative brain weights (n=5/sex)				
Weights (g)	**0 mg/kg**	**150 mg/kg**	**500 mg/kg**	**1500 mg/kg**
Males				
Body wt	307.8±18.2	312.2±15.7	303.5±15.2	289.2±10.6
Brain wt	1.95±0.04	1.93±0.09	1.95+0.04	1.92±0.07
Brain/body wt (%)	0.64±0.05	0.62±0.02	0.64±0.04	0.66±0.02
Female				
Body wt	203.4±6.1	200.2±14.4	206.6±14.3	189.6±17.9
Brain wt	1.77±0.04	1.75±0.06	1.76±0.05	1.77±0.03
Brain/body wt (%)	0.87±0.04	0.88±0.07	0.85±0.06	0.94±0.10

Data were extracted from page 81, MRID 45357501.
* Statistically different ($p < 0.05$) from the control.

3. **Neuropathology:** No treatment-related neuropathological alterations were observed (Table 6). The only findings were demyelination of the sciatic and tibial nerves seen in one control group male and demyelination of the sciatic nerve in one high-dose female.

TABLE 6. Incidence of neuropathological findings				
Lesion	**Male**		**Female**	
	0 mg/kg	**1500 mg/kg**	**0 mg/kg**	**1500 mg/kg**
Sciatic Nerve Demyelination (Total) minimal	1	0	0	0
Tibial Nerve Demyelination (Total) minimal	1	0	0	0

n=5 for all groups
Data were extracted from pages 85-86, MRID 45357501.

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that acetochlor did not produce any evidence of a direct or specific effect on the nervous system at any of the dose levels tested. They considered the effects observed in the FOB and on locomotor activity to be related to general toxicity attributable to acetochlor administration and concluded that the NOAEL was 500 mg/kg bw. To support their conclusion, they quoted the guidelines that state that "the relevance of statistically significant test results from an FOB is judged according to the number of signs affected, the dose(s) at which effects are observed, and the nature, severity, and persistence of the effects and their incidence

in relation to control animals. In general, if only a few unrelated measures in the FOB are affected, or the effects are unrelated to dose, the results may not be considered evidence of a neurotoxic effect. If several neurological signs are affected, but only at the high dose and in conjunction with other overt signs of toxicity including systemic toxicity, large decreases in body weight, decreases in body temperature, or debilitation, there is less persuasive evidence of a direct neurotoxic effect."

B. **REVIEWER COMMENTS:** Effects of acetochlor treatment were limited to the highest dose tested (1500 mg/k bw). Effects observed at this dose level were mortality (one male death that is attributed to treatment), reduced body weights and body weight gain in both sexes, reduced food consumption in both sexes, increased clinical signs and effects observed during the FOB in both sexes, and decreased locomotor activity in females. The NOAEL for these effects is 500 mg/kg bw.

Although this reviewer believes that the potential neurotoxic effects observed during the FOB and during motor activity testing are likely not a direct effect of acetochlor on the nervous system *per se* (no effect on brain weights or neuropathology), it is difficult to definitively separate neurotoxicity and systemic toxicity. Therefore, this reviewer cannot concur with the investigators that the effects observed were solely attributable to systemic toxicity. In addition, the decreases in body weights and body weight gains observed at the high dose were not large, only one animal died, and none of the other animals on study became debilitated because of treatment.

Based on the effects seen in this study, the LOAEL was 1500 mg/kg bw/day based on decreased body weights and body weight gain (males and females), reduced food consumption (males and females), increased incidence of clinical signs during the FOB, and decreased motor activity in females at the time of peak effect, with a NOAEL of 500 mg/kg bw/day.

C. **STUDY DEFICIENCIES:** Methods and equipment used for the quantitative measures during the FOB were not described. This deficiency does not impact the acceptability of the study.

EPA Reviewer: Ayaad Assaad, D.V.M., Ph.D. **Signature:**
Risk Assessment Branch IV Health Effects Division (7509P) **Date:** Dec 3, 2015
EPA Secondary Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** Dec. 3, 2015
Template version 09/11

TXR#: 0057234

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity, OPPTS 870.6200b [§82-7] [feeding]-[rat]; (No OECD guideline).

PC CODE: 121601 **DP BARCODE:** D274337

TEST MATERIAL (PURITY): Acetochlor, tech (94.7% a.i.)

SYNONYMS: 2-chloro-N-ethoxymethyl-6'-ethylacet-o-toluidide; trade names include Harness, Sacemid, Acenit, Surpass, TopNotch and Trophy.

CITATION: Kilgour, J.D. (2001) Acetochlor: Subchronic Neurotoxicity Study in Rats. Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, UK. Study No. CTL/PR1176. February 8, 2001. MRID 45357502. Unpublished report.

SPONSOR: Acetochlor Registration Partnership, c/o Monsanto Co., St. Louis, MO.

EXECUTIVE SUMMARY: In an oral subchronic neurotoxicity study (MRID 45357502), Acetochlor (tech., 94.7% a.i., batch/lot # P11) was administered in the diet to 12 Alpk:APfSD rats/sex/group at dose levels of 0, 200, 600 or 1750 ppm (equivalent to 0, 15.4, 47.6 or 139.0 mg/kg bw/day, males and 0, 18.3, 55.9 or 166.5 mg/kg bw/day, females) for 93 days. A neurobehavioral assessment (functional observational battery and motor activity testing) was performed in all animals/sex/group at -1 week pretest and at weeks 2, 5, 9 and 14. At study termination, 5 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Brain, spinal cord and peripheral nervous system of the control and high dose animals were examined microscopically; brain weights were also measured.

At 1750 ppm, slight but statistically significant decreases in mean body weight (2.6 to 4.1% less than controls) and weight gain (↓14 to ↓20%, males and ↓25% to ↓30%, females) were reported in both sexes in the early weeks of the study. Decreases thereafter were not significant but continued throughout the study and at termination, mean body weight/weight gain was decreased by 5.4%/11.3% in males and 3.3%/9.8% in females at study termination. During the FOB evaluations at week 2, but not at later times, a statistically significant decrease in hindlimb grip strength (↓44%) in males was observed, these decreases in FOB in week-2 were not considered treatment related since it was not detected in weeks 5, 9 or 14 or in females at any dose level or any measurement interval. There were no treatment-related

increases in clinical signs of toxicity nor effects on other neurobehavioral parameters in the FOB, motor activity, brain weight or gross/microscopic neuropathology. **The LOAEL is not observed. The NOAEL is 1750 ppm (139.0 mg/kg, the highest dose tested).**

The study is classified as Acceptable (Guideline) – and satisfies the guideline requirements for a subchronic neurotoxicity study in rats (870.6200b).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	Acetochlor
Description:	Technical grade, light brown liquid.
Lot/Batch #:	P11 (CTL test substance reference no. Y06341/110)
Purity:	94.7% a.i.
Compound Stability:	Stable stored at room temperature in the dark.
CAS # of TGAI:	34256-82-1

2. Vehicle and/or positive control: None (mixed directly into diet as premix)

3. Test animals:			
Species:	Rat		
Strain:	Alpk:APfSD (Wistar-derived)		
Age/weight at study initiation:	Approximately 42 days at study start. Weight range 220-278 g, males and 159-206 g, females.		
Source:	Rodent Breeding Unit, Alderly Park, Macclesfield, Cheshire, UK		
Housing:	Group housed (4-5/cage). Type of cage not specified in report.		
Diet:	CT1, Special Diet Services Limited, Stepfield, Witham, Essex, UK, *ad libitum*		
Water:	Local tap water *ad libitum*		
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22±3 °C 30-70% 15/hr 12 hrs dark/12 hrs light	
Acclimation period:	at least 5 days		

B. STUDY DESIGN:

1. In life dates - Start: January 18, 2000, start of pretest period (January 25, 2000 - start of test material administration). End: July 13, 2000.

2. Animal assignment and treatment - Animals were randomly assigned to the test groups noted in Table 1 (stratified by body weight and eliminating any rats that did not respond to a tail-flick test prior to the randomization procedure). Test substance was administered in the diet for 93 days. The mg/kg/day values were calculated based on the nominal dietary levels of acetochlor in the test diets. The test groups were further divided into six single-sex replicate groups for conducting the neurobehavioral assessments and perfusion/sacrifice.

Table 1. Study Design

Experimental Parameter	Dose Group, ppm (mg/kg bw/day)			
	0	200 15.4 ♂/18.3 ♀	600 47.6 ♂/55.9 ♀	1750 139.0 ♂/166.5 ♀
Total number of Animals/sex/group	12/sex	12/sex	12/sex	12/sex
Behavioral Testing (FOB, Motor Activity)	12/sex	12/sex	12/sex	12/sex
Neuropathology	5/sex	5/sex[1]	5/sex[1]	5/sex
Blood cholinesterase determination	ND	ND	ND	ND
Brain cholinesterase determination	ND	ND	ND	ND

[1] The retained tissues from all perfused animals from the control and high dose groups were examined microscopically. Low and mid dose animals were processed for microscopic evaluation by *in situ* perfusion, but tissues were not examined. ND=Not determined.

Dose levels were chosen based on the findings of a 28-day dietary range-finding study (Study No. PR1182), summarized briefly in Appendix H, p. 138 of the study report. Eight Alpk:AP_fSD rats/sex/dose group were administered acetochlor in their diet at concentrations of 0, 1250, 1750 or 2250 ppm (mg/kg/day values were not provided in the summary) for a total of 28 days. Animals were evaluated for clinical signs of toxicity/mortality, bodyweight and food consumption. These parameters were observed daily up to Day 8, then weekly thereafter until termination on Day 29. Neurological screening evaluations (FOB, motor activity assessments) were not performed.

No effects were reported at 1250 ppm. At 1750 ppm, mean bodyweight/bodyweight gain were reduced by 5%/12% (males) and 2%/4% (females) relative to controls. Bodyweight/weight gain at 2250 ppm were reduced by 7%/16% (males) and 2%/6% (females). Mean food consumption values were statistically significantly lower in males on Day 1 but not at later times, and were also lower in females (not significant) through Day 4 but not at later times. The study author stated that a dose-response was not observed, but no quantitative values were provided. No clinical signs of toxicity were reported at any dose level.

In addition to the range-finding study, the study authors cited the 2-generation reproductive toxicity study in rats (MRID 45357503); in which diminished body weight gain of 19% below controls was observed in females of the 1750 ppm group at Week 11, as support for the dose selection. Independent inspection of results in the multi-generation reproduction confirms the study author's citation on the body weight gain deficit among females (F_0 generation, premating) at 1750 ppm. Based on these findings, the study authors considered 1750 ppm to be an appropriate high dose.

3. <u>Test Substance Preparation and Analysis</u>:

Diets were prepared in 30 kg batches. A concentrated premix was first prepared by mixing appropriate

amounts of test substance (adjusted for purity) with 1 kg CT1 diet, then combining the premix with 29 kg of diet in a Pharma Matrix Blender (T.K. Fielder). Diets were aliquoted into glass jars and stored frozen until needed. Frequency of preparation of diets was not indicated. Once thawed, any remaining diets were discarded after one week. Homogeneity was evaluated in duplicate samples taken from the top, middle and bottom of the 200 and 1750 ppm diets prior to the start of dosing (sampling on 1/21/00). The stability testing was performed in an earlier multi-generation reproductive toxicity study in rats (results summarized in this report). During the study, samples from the initial batch of treated food were analyzed for concentration of acetochlor once prior to the study start. The methods section (4.2, p. 16 of study report) stated that an additional analysis was performed on all diet preparations once during the study; however, only pretest analyses of the 600 and 1750 ppm diets were presented in this study report. A total of four analyses on 200 ppm diets were performed due to high values obtained in the initial analysis (two analyses of the initial 200 ppm diet preparation and subsequent analyses of two additional batches). All dietary analyses were performed using gas chromatography.

Results -

Homogeneity Analysis: The study author calculated the deviations of the mean concentrations at each sampling point (mean of duplicate samples) from the overall concentration means of the 200 and 1750 ppm diets to be within 5%. The overall mean for the first batch of 200 ppm diet concentration was 253 ppm; the individual samples ranged from 241 to 262 ppm. Since this concentration exceeded the intended 200 ppm level, a replacement 200 ppm batch was prepared January 28, the day of initiation of the in-life phase of the study. The overall mean for the second batch of 200 ppm diet was 214 ppm (individual samples ranged from 204-225 ppm) (Table 3, p. 47 of the study report). The overall mean for the 1750 ppm diet was 1712 ppm (the individual samples ranged from 1613 to 1772 ppm).

Stability Analysis: The stability analysis of acetochlor in the multi-generation reproductive toxicity study (MRID 45357503) demonstrated that the test material was stable in the diet for at least 2 months when stored at -20 ^{0}C. After the initial analysis was performed, mean analytical concentrations ranged from 95.6% to 103% of target (200 ppm diets) and 96.2% to 102.1% of target (1750 ppm diets).

Concentration Analysis: In the concentration analysis, values for the initial preparation of 200 ppm diet ranged from 241 ppm to 262 ppm (mean 253 ppm, 126.5% of nominal concentration) (Table 3, p. 46 of study report). When reanalyzed, a mean concentration of 218 ppm (109%) was obtained. The second preparation (1/28/00) ranged from 205-246 ppm and had a mean concentration of 221 ppm (110.5% of target). Subsequent low dose preparations (prepared 2/17/00 and 3/27/00) had means of 211 ppm and 185 ppm (105.5% and 92.5% of nominal concentration) (Table 1, p. 44 of study report). The 600 ppm and 1750 ppm diets prepared on 1/21/00 were all within less than 10% of the nominal concentration (mean 595 or 99.2% of nominal; individual samples 585 ppm and 605 ppm, mid dose; mean 1712 ppm; range 1613 to 1770 ppm, high dose).

The analytical data indicated that the mixing procedure was adequate, the test material was stable in the diet and, with the exception of the early low dose diets, the variation between nominal and

actual dosage to the animals was acceptable. The variations observed in the low dose diet preparations are not considered to have adversely affected the integrity of this study.

4. Statistics - Weekly food consumption and food efficiency (both for weeks 1-4, 5-8, 9-13 and 1-13), time to tail-flick, landing foot splay and forelimb/hindlimb grip strengths were analyzed using ANOVA. Total motor activity data were analyzed using a repeated-measures ANOVA. Motor activity habituation was assessed visually, rather than statistically. Body weight data were analyzed using ANCOVA on initial (week 1) bodyweights. Brain weight data were analyzed by ANOVA and ANCOVA on the final bodyweight. All statistical analyses except for motor activity were performed using the MIXED procedure in SAS. The least-squares means for each group were determined using the LSMEAN option in SAS PROC MIXED. Differences from control were obtained by comparing the least-squares means of each treatment group and the control group and statistical analysis performed using a two-sided Student's t-test based on the error mean square in the analysis.

C. METHODS/OBSERVATIONS:

1. Mortality and Clinical Observations: Animals were observed daily for mortality and moribundity. Detailed clinical observations were recorded at the same time that the weekly body weights were recorded.

2. Body weight: Animals were weighed at 1 week pretest, on day 1 just prior to initial administration of test diet and then weekly thereafter through termination at Week 13.

3. Food consumption: Food consumption was measured per cage and calculated as g food/animal/day at weekly intervals. Food efficiency was calculated as g bodyweight gain of rats in cage per 100 g consumed food.

4. Cholinesterase Determination: Cholinesterase activity was not determined.

5. Ophthalmological Examination: The eyes of all animals were examined for ophthalmologic abnormalities prior to the initiation of the study. At study termination, control and high dose animals were examined ophthalmologically.

6. Neurobehavioral Assessment:a. Functional Observational Battery (FOB): Standard FOB testing was conducted on all animals in the study at weeks -1, 2, 5, 9 and 14. The parameters that were evaluated are shown below. One technician acted as the observer and noted the findings for each animal (observations were blind with respect to treatment group) and a second technician recorded the findings directly into a computer data recording system (pp. 19-22 of study report). The duration of the open field observation period was not indicated. Details of the scoring criteria for FOB parameters, duration of observation periods and testing equipment and methods used for grip strength, foot splay and tail flick measurements were not provided in the study report.

The CHECKED (X) parameters were examined:

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*	X	Mobility
	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
X	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
X	Feces consistency	X	Respiratory rate+	X	Urination / defecation*
		X	Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*		Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behavior*
X	Startle response*			X	Backing
X	Pain response* (toe pinch)				Time to first step
X	Pupil response*				
X	Eyeblink response (corneal)		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
X	Forelimb extension	X	Body weight*		Hindlimb extensor strength
X	Hindlimb extension (characterized as limb function for both hind and forelimb)	X	Body temperature+ (recorded only as hypothermia/hyperthermia; not measured quantitatively)	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
					Rotarod performance

*Required parameters; +Recommended parameters. Most of the parameters listed under "Home Cage Observations" in this study were actually examined in the open field - this does not affect the interpretation of the study findings.

b. Locomotor Activity: Motor activity was evaluated at Weeks -1, 2, 5, 9 and 14. The study report did not indicate when the motor activity testing was conducted in relation to FOB performance testing. Animals were placed in a stainless steel cage of unspecified size and small and large movements were recorded as an activity count using a Coulbourn Lab Linc Infra-red Motion Activity System. Each session began immediately after the animal was placed in the cage and consisted of ten five-minute sub-sessions. The treatment groups were counter balanced for test times and across devices to avoid bias. No details about the testing environment (e.g., soundproofing, subdued lighting, etc.) were provided.

7. Sacrifice and Pathology:

At study termination (Week 14), five animals/sex/group were sacrificed by barbiturate anesthesia (intraperitoneal injection), followed by perfusion fixation using modified Karnovsky's fixative. Brains were removed and weighed, then cut into transverse sections at 9 levels. Although the study report did not state the specific regions of the brain that were examined microscopically, it may be assumed that each of the brain structures listed in the table below were evaluated since 9 sections were examined. All of the tissues preserved for light microscopy, listed below, were examined in the control and high dose groups. These tissues were examined macroscopically for grossly visible abnormalities for all of the dose groups.

The CHECKED (X) tissues were evaluated.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Proximal
X	Center of cerebrum		
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian Ganglion	X	Cervical dorsal root fibers
	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve		
X	Eyes		
X	Gastrocnemius muscle		

8. Positive Controls: Positive control data were provided to validate the procedures and observers of the performing lab to detect the effect of chemicals on FOB parameters, motor activity, behavior, neuropathological lesions, and other parameters indicative of neurotoxicity. These data were obtained from MRIDs #45811002 & 45811003 (December 2000). Male and female rats dosed with l0 mg **amphetamine sulphate**/kg (I.P) showed the following clinical signs; increased activity in 10 males and 10 females, bizarre behavior in 2 males and 2 females, diarrhea in 2 males, hypothermia in 1 male and 1 female, piloerection in 9 males and 10 females, salivation in 9 males and 10 females, signs of urinary incontinence in 1 male and urinary incontinence in 8 males and 9 females. Decreased forelimb and hindlimb strength was also observed in male rats.

Morphine sulphate (100 mg/kg bw; single gavage dose; MRID 45811003) induced increased time to tail flick in both sexes (i.e. a decrease in sensory perception), and decreased forelimb grip strength in females.

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs - No treatment-related clinical signs of toxicity were reported. A few signs such as scabbing and bulging eyes were observed occasionally in high dose animals but were not considered to be treatment-related due to the low incidence.

2. Mortality - All of the animals survived until termination.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Mean body weight and weight gain values at selected times during the study are provided below in Table 2:

Table 2. Body weight and cumulative body weight gain (g ± s.d.)

Observation (g ± s.d.)		Dose Level, ppm (mg/kg bw/day)			
		0	200 15.4 ♂/18.3 ♀	600 47.6 ♂/55.9 ♀	1750 139.0 ♂/166.5 ♀
Body weight-Males					
Week 1	Mean	249.8 ± 15.3	251.7 ± 11.9	248.6 ± 14.3	247.3 ± 18.8
2	Mean	299.2 ± 16.6	301.8 ± 12.9	296.3 ± 16.9	287.0 ± 23.3
	Adjusted Mean	298.6	299.2	297.1	289.2** (-3.1%)
3	Mean	327.8 ± 20.2	333.7 ± 14.7	330.3 ± 19.7	314.8 ± 28.3
	Adjusted Mean	327.2	330.9	331.2	317.2*(-3.1%)
4	Mean	352.3 ± 23.2	361.8 ± 19.4	362.1 ± 23.9	340.2 ± 34.2
	Adjusted Mean	351.6	358.7	363.1	342.9 (-2.5%)
8	Mean	437.6 ± 33.6	445.8 ± 30.9	449.8 ± 33.9	412.5 ± 44.8
	Adjusted Mean	436.8	442.2	451.1	415.7 (-4.8%)
11	Mean	477.0 ± 38.9	494.3 ± 37.0	489.1 ± 40.9	449.0 ± 48.3
	Adjusted Mean	476.2	490.6	490.3	452.3 (-5.0%)
14	Mean	500.3 ± 42.4	524.0 ± 40.0	516.0 ± 44.1	469.3 ± 52.7
	Adjusted Mean	499.5	520.1	517.3	472.7 (-5.4%)
Body weight-Females					
Week 1	Mean	180.0 ± 11.7	180.7 ± 13.2	177.5 ± 7.9	180.4 ± 15.7
2	Mean	196.8 ± 13.9	198.6 ± 17.3	196.1 ± 10.1	192.0 ± 17.0
	Adjusted Mean	196.4	197.5	198.4	191.2* (-2.6%)
3	Mean	212.3 ± 16.3	213.8 ± 18.8	209.3 ± 13.1	204.7 ± 19.8
	Adjusted Mean	211.8	212.6	211.8	203.8* (-3.8%)
4	Mean	223.3 ± 16.7	227.3 ± 21.7	223.4 ± 13.9	214.8 ± 23.5
	Adjusted Mean	222.9	225.9	226.2	213.8* (-4.1%)
8	Mean	250.1 ± 17.5	256.7 ± 23.7	253.0 ± 18.2	239.2 ± 27.3
	Adjusted Mean	249.6	255.3	255.9	238.1 (-4.6%)
11	Mean	259.1 ± 13.9	267.8 ± 28.4	263.8 ± 19.8	254.5 ± 29.6
	Adjusted Mean	258.6	266.4	266.6	253.5 (-2.0%)
14	Mean	270.0 ± 19.5	279.8 ± 28.0	274.5 ± 21.0	261.6 ± 28.9
	Adjusted Mean	269.5	278.5	277.3	260.6 (-3.3%)
Body weight gain-Males					
Week 2		49.3 ± 4.5	50.1 ± 4.5	47.7 ± 6.1	39.7 ± 8.4** (-19.5%)
3		77.9 ± 8.5	82.0 ± 7.8	81.7 ± 11.9	67.4± 15.4* (-13.5%)

Table 2. Body weight and cumulative body weight gain (g ± s.d.)

Observation (g ± s.d.)	Dose Level, ppm (mg/kg bw/day)			
	0	200 15.4 ♂/18.3 ♀	600 47.6 ♂/55.9 ♀	1750 139.0 ♂/166.5 ♀
4	102.4 ± 11.8	110.2 ± 15.3	113.5 ± 15.9	92.8 ± 21.0 (-9.4%)
8	187.8 ± 23.3	194.2 ± 30.3	201.3 ± 26.5	165.2 ± 32.4 (-12.0%)
11	227.2 ± 29.7	242.7 ± 36.9	240.5 ± 33.8	201.7 ± 37.1 (-11.2%)
14	250.4 ± 35.1	272.3 ± 39.7	267.4 ± 36.3	222.0 ± 42.5 (-11.3%)
Body weight gain-Females				
Week 2	16.8 ± 7.5	17.9 ± 5.9	18.6 ± 6.4	11.6 ± 5.0* (-31%)
3	32.3 ± 10.6	33.1 ± 10.7	31.8 ± 10.7	24.3 ± 5.3* (-24.8%)
4	43.3 ± 13.4	46.6 ± 13.8	45.9 ± 10.5	34.3 ± 10.7 (-20.8%)
8	70.1 ± 13.3	76.0 ± 16.7	75.5 ± 15.1	58.8 ± 15.3 (-16.1%)
11	79.1 ± 13.7	87.1 ± 21.3	86.3 ± 17.3	74.1 ± 18.2 (-6.3%)
14	90.0 ± 17.6	99.2 ± 22.3	97.0 ± 18.6	81.2 ± 17.2 (-9.8%)

Data were extracted from Table 5A (pp. 56-59) and Table 5B (pp. 60-61) of MRID 45357502. N = 12, all groups.
Values represent mean ± s.d.
*=$p<0.05$, **=$p<0.01$, when compared to control means.

In males, statistically significant decreases in mean body weight were observed during weeks 2 and 3 of the study (3.1% below controls, mean adjusted body weights). Thereafter, body weights remained slightly lower than controls but statistical significance was not identified. By Week 14, mean adjusted body weight was 5.4% below controls. Cumulative body weight gain was significantly reduced during weeks 2 and 3 (19.5% and 13.5% less than controls), but no statistical significant decreases were observed at subsequent times. By week 14, gain was reduced by 11.3%.

Statistically significant decreases in mean body weight (adjusted) were also observed in females during weeks 2, 3 and 4 (3.5%, 3.8% and 4.1% below controls). Similar decreases were observed throughout the study but were not statistically significant. At Week 14, mean body weights were 3.1% below controls. Body weight gain was significantly decreased during Weeks 2 and 3 34.5% and 24.8% less than controls), remaining slightly lower throughout the study but not significant (at Week 14, 10% below controls).

The decreases in body weight observed in both sexes were small and statistically significant only in the early weeks of the study, but body weights remained slightly reduced throughout the study and were observed in both sexes. Decreased body weight/weight gain were also reported in the range-finding study and the 2-generation reproductive toxicity study (see rationale for dose selection, above).

Although the decreases observed in this study were marginal, TB agreed with the study author that they were treatment-related.

C. FOOD CONSUMPTION: Food consumption (g/animal/day) values for selected time points are shown below in Table 3:

Table 3. Food consumption (g/animal/day)

Week No.	Dose Level, ppm (mg/kg bw/day)			
	0	200 15.4 ♂/18.3 ♀	600 47.6 ♂/55.9 ♀	1750 139.0 ♂/166.5 ♀
Males				
Week 1	34.0 ± 0.7	33.7 ± 1.0	33.3 ± 0.6	32.5 ± 1.4
2	33.3 ± 0.9	32.8 ± 1.7	33.6 ± 0.9	31.7 ± 1.2*
3	31.2 ± 0.9	31.4 ± 3.1	33.4 ± 1.1	30.7 ± 1.2
6	30.2 ± 0.6	30.8 ± 1.4	31.2 ± 0.8*	28.8 ± 0.4*
10	31.5 ± 1.2	31.7 ± 2.0	32.1 ± 1.0	29.7 ± 0.4*
13	30.9 ± 2.3	30.9 ± 1.9	31.5 ± 1.3	29.1 ± 0.6*
Females				
Week 1	23.3 ± 1.4	23.9 ± 1.3	23.5 ± 1.0	24.1 ± 1.5
2	22.8 ± 0.9	23.0 ± 1.5	23.1 ± 1.4	23.2 ± 2.6
3	22.6 ± 1.2	23.4 ± 1.1	22.8 ± 0.7	23.7 ± 1.1
6	21.5 ± 0.2	21.8 ± 0.8	22.1 ± 0.5	21.9 ± 0.7
10	21.5 ± 0.5	21.8 ± 1.5	22.4 ± 0.8	22.8 ± 2.1
13	21.5 ± 1.2	21.8 ± 1.4	21.5 ± 1.2	19.8 ± 0.3

Data were extracted from Table 6, pp. 62-65 of MRID 45357502. N = 3 (each N represents one cage of 4 animals/sex).
Values represent mean ± s.d.
*=p<.05, **=p<.01, when compared to control means

Animals were not housed individually in this study; therefore food consumption values represent an average calculated from cage group food consumption. In males, sporadic statistically significant decreases in mean food consumption (expressed as g/animal/day) were observed but consumption was

within -10% of controls. When adjusted for the slightly reduced mean body weights observed at the high dose, food consumption was not decreased (data not shown). No treatment-related effects on food consumption were observed in females.

Food efficiency was also calculated for intervals of Weeks 1-4, 5-8 and 9-13, but not for individual weeks. For males, a statistically significant reduction in food efficiency was observed between weeks 5-8 (11.3% less than controls) in high dose males and values at high dose tended to be slightly lower (overall weeks 1-13 efficiency was -7.1% below controls). A statistically significant increase in efficiency was also observed during weeks 5-8 at 200 ppm (12% above controls). There were no treatment-related statistically significant changes in food efficiency in females, although values at the high dose were generally marginally lower than controls (overall weeks 1-13 efficiency was 9.7% less than controls). The data suggest a slight reduction in food efficiency; however, these calculations are considered of limited value because they do not provide information about efficiency in individual animals in this study due to group housing.

D. NEUROBEHAVIORAL RESULTS

The only statistically significant finding observed in the neurobehavioral studies was decreased hindlimb grip strength in the males at Week 2. These data are presented below for all of the sampling weeks in Table 4:

1. FOB Findings:

Table 4. Functional observational battery results

Observation	Dose Level, ppm (mg/kg bw/day)			
	0	200 15.4 ♂/18.3 ♀	600 47.6 ♂/55.9 ♀	1750 139.0 ♂/166.5 ♀
Males				
Hindlimb grip strength, g				
Pretest	413 ± 56	440 ± 69	435 ± 79	423 ± 52
Week 2	621 ± 127	531 ± 160	565 ± 113	348 ± 117**
Week 5	742 ± 190	696 ± 140	758 ± 135	696 ± 140
Week 9	752 ± 355	731 ± 67	596 ± 105	615 ± 148
Week 14	1056 ± 174	1098 ± 158	1158 ± 136	1060 ± 184

	Dose Level, ppm (mg/kg bw/day)			
Test Week	**0**	**200** **15.4 ♂%/18.3 ♀**	**600** **47.6 ♂/55.9 ♀**	**1750** **139.0 ♂/166.5 ♀**
Females				
Hindlimb grip strength, g				
Pretest	425 ± 53	406 ± 88	406 ± 81	377 ± 58
Week 2	377 ± 72	325 ± 81	373 ± 82	379 ± 97
Week 5	521 ± 130	546 ± 108	442 ± 157	500 ± 131
Week 9	398 ± 71	373 ± 106	388 ± 109	440 ± 113
Week 14	740 ± 150	781 ± 189	742 ± 184	740 ± 129

Data were extracted from Table 11, pp. 102-103, MRID 45357502. N = 12, all groups.
Values represent strength of hindlimb grip as measured in g.
** $p<0.01$ compared with controls

At 1750 ppm, a statistically significant decrease of 44% below controls was observed for hindlimb grip strength in males at Week 2. No significant decreases were observed at the subsequent testing times. Forelimb grip strength was not decreased by treatment. There were no significant changes in hindlimb or forelimb grip strength in females at any testing time during this study. The significance of this decrease is unclear since a consistent or progressive decrease in this parameter was not observed in this study and females were not affected. The decrease may have been in part secondary to decreased body weight gain relative to the other groups, or a random variation. There appeared to be some variation in measurements - for example, at week 9, grip strength of females in all the groups was slightly lower than Week 5 instead of the same or slightly greater. However, the absence of this response in females and in other measurement intervals at weeks 5, 9 and 14, ruled out this effect as treatment-related. There were no treatment-related effects on other parameters evaluated in the FOB.

2. **Motor activity:** Motor activities (total) during the study are shown below in Table 5:

Table 5. Motor activity assessment (total activity counts for 50-minute session)

Test Week	Dose Level, ppm (mg/kg bw/day)			
	0	200 15.4 ♂/18.3 ♀	600 47.6 ♂/55.9 ♀	1750 139.0 ♂/166.5 ♀
Males				
Pre-test (-1)	325.9 ± 171.8	319.2 ± 136.8	382.9 ± 191.2	325.5 ± 190.0
Week 2	423.7 ± 163.1	491.0 ± 122.0	513.7 ± 142.7	513.3 ± 138.0
Week 5	613.7 ± 171.0	647.9 ± 157.2	599.3 ± 162.0	616.6 ± 154.9
Week 9	504.5 ± 184.6	540.7 ± 130.4	454.3 ± 124.4	521.7 ± 157.2
Week 14	420.1 ± 159.0	461.3 ± 156.3	375.8 ± 116.2	392.4 ± 124.9
Females				
Pre-test (-1)	409.0 ± 160.1	457.8 ± 156.2	444.0 ± 203.8	367.2 ± 199.7
Week 2	659.4 ± 158.1	674.0 ± 88.9	700.6 ± 92.1	629.5 ± 163.3
Week 5	785.0 ± 69.4	782.2 ± 50.3	796.4 ± 75.3	727.6 ± 71.4
Week 9	739.8 ± 100.4	790.1 ± 87.5	783.3 ± 69.5	717.6 ± 104.0
Week 14	742.5 ± 108.2	745.8 ± 90.7	752.3 ± 99.3	728.3 ± 116.6

Data were extracted from Table 13 (pp. 106-107), MRID 45357502. N = 12, all groups. Values represent mean ±s.d.

There were no treatment-related changes in motor activity observed during this study.

A comparison of the sub-session interval activity measurements for each testing week also indicated no significant differences among the dose groups for either sex (data not shown in this DER). Comparison of the activities in the sub-sessions indicated that males showed habituation (reduction and leveling of activity in later sub-sessions due to acclimation to environment) in all of the testing sessions. Although the pretest (week -1) MA evaluation of the females in all treatment groups showed some habituation, the females in all of the treatment groups tended to have similar activities throughout the sub-sessions during Weeks 2, 5, 9 and 14. The reason for this apparent lack of habituation is unclear. Since no treatment-related effects on motor activity were observed in males and similar total and sub-session motor activities were observed in all groups of females, no further studies are required at this time to address this issue.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** No treatment-related gross findings were observed in a comparison of the control

and high dose animals (Table 17, pp.123-124 study report).

2. Brain weight: No treatment-related changes in mean brain weights were observed in a comparison of the control and high dose animals (Table 16, p. 121 study report).

3. Neuropathology: There were no treatment-related microscopic neuropathological lesions observed in high dose group animals. The incidence of tibial nerve demyelination (minimal) was detected in 2/5 males (40%) and 1/5 females (20%), compared to 0/5, in all other groups. This finding was probably sporadic and of minimal severity and is commonly observed in untreated rats in subchronic studies. Minimal demyelination of the sciatic and sural nerves of control females (1/5 each vs. 0/5, all other groups) was also observed. Historical control data for these lesions from this laboratory were not provided but are not considered necessary for interpretation of this finding (Table 18, pp. 125-126 study report).

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author determined that there was no evidence of neurotoxicity (neurobehavioral or neuropathological findings) following administration of acetochlor in the diet up to 1750 ppm for 93 days. A statistically significant decrease in hindlimb grip strength in high dose males at Week 2, but not at later times, was considered sporadic but possible explanations for this transient change were not provided. Slight but statistically significant decreases in mean body weight and weight gain were reported for both males and females in the early weeks of treatment and values remained slightly lower than controls throughout the study. These decreases were not considered treatment-related because it was detected only in males and only at week-2 of the study, but insignificant as of its effect on the outcome of the study. There was no evidence of toxicity at 200 or 600 ppm. The study authors considered 1750 ppm to be the LOAEL for this study, based on the decreased body weight/weight gain observed in both sexes, and the NOAEL 600 ppm.

B. REVIEWER COMMENTS: The reviewers agreed with most of the conclusions of the study author but whereas the study authors considered the statistically significant but transient decrease in hindlimb grip strength in males likely to be incidental. However, absence of this response in females and in other measurement intervals at weeks5, 9 and 14, ruled out this effect as treatment-related. There were no treatment-related effects on other parameters evaluated in the FOB.

The decreases in mean body weight observed in males and females were small and largely due to lower gain in the early weeks. These effects are minor and insignificant, even if they were true and has no impact on the outcome of the study. Although the animals could probably have tolerated dose levels somewhat higher than1750 ppm, further testing is not required because a marginal level of toxicity was achieved.

EPA reviewers concluded that there were no treatment-related increases in clinical signs of toxicity nor effects on other neurobehavioral parameters in the FOB, motor activity, brain weight or gross/microscopic neuropathology. **The LOAEL is not observed. The NOAEL is 1750 ppm (139.0 mg/kg).**

The study is classified as Acceptable (Guideline) – and satisfies the guideline requirements for a subchronic neurotoxicity study in rats (870.6200b).

C. **STUDY DEFICIENCIES:** None.